CAROTID PLAQUE TISSUE DIFFERENTIATION BASED ON RADIOFREQUENCY ECHOGRAPHIC SIGNAL LOCAL SPECTRAL CONTENT (RULES: Radiofrequency Ultrasonic Local EStimators)

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ABSTRACT

An echographic method is proposed in order to detect and differentiate carotid vessel plaques. The method based on a novel spectral processing procedure for the radiofrequency echo signal is named RULES (Radiofrequency Ultrasonic Local EStimators). It allows the extraction of local spectral parameters related to the organization and mechanical properties of an investigated tissue region. Spectral images are produced through a processing procedure, based on a statistical analysis of radiofrequency signal spectral coefficients, calculated with the Discrete Wavelet Packet Transform. For each organ, the regions of the investigated tissue, which exhibit the same distribution of the spectral coefficients, were considered homogeneous and were put in correspondence with local tissue condition (healthy or with pathology), as derived from histological analysis.

Index Terms— Radiofrequency echo signal, spectral processing, tissue differentiation

1. INTRODUCTION

Recently, many authors [1]-[5] have agreed that carotid artery stenosis degree estimation is not sufficient to evaluate the clinical risk for patients. As a consequence, further investigations of carotid plaque structure and morphology are necessary. Nowadays arteriography, CT-angio, Color Doppler, Ultrasonography and Magnetic Resonance represent the main imaging techniques - used to determine the severity of carotid atherosclerosis and the need for endartectomy. Among them, ultrasonography is preferred because it is minimally invasive and relatively inexpensive.

The most important ultrasonic information for carotid plaque characterization is that derived from the plaque surface and internal organization [6]-[7]. A plaque surface appears as a regular interface when its border is smooth and unbroken, while an irregular profile is observed when ulceration or a break of the fiber cap occur. The presence of ulceration inside the plaque structure can represent a high risk factor for stroke. As a consequence, an accurate evaluation of plaque structure is of primary importance.

In this paper, a novel investigation method named RULES (Radiofrequency Ultrasonic Local EStimators) is presented for carotid plaque tissue differentiation. The method is based on the extraction of local spectral content from the radiofrequency (RF) echo signal. The experimental work has been organized in two different phases: *in vivo* acquisition before surgical operation and *in vitro* acquisition on the removed specimens.

When a new organ must be investigated with RULES a learning phase has to be conducted in order to tailor the most appropriate set of RULES algorithm parameters.

The learning phase experimental protocol scheduled a postoperative histopathological examination of the removed specimen in order to produce histological maps to evaluate the presence and the extension of calcifications, fibrous tissue, necrosis and fiber cap.

The learning phase for carotid characterization was performed on 187 *in vitro* echographic frames, supplied with their related 187 histological maps.

Different kinds of structural organizations were recognized and mapped with different chromatic code over the B-Mode image. Then, by comparing the homogeneous image portions so produced with the corresponding zone of the histological maps, it was possible to drive the search of the most appropriate set of RULES parameters ("Configuration") for plaque structure differentiation. The learning phase was considered to be concluded when a perfect correspondence occurred between RULES and histology. By using this procedure, two "Configurations" were defined and were then tested on the *in vivo* acquisitions previously collected.

The RULES processing and data visualization procedure is supported by a hardware-software platform, FEMMINA (Fast Echographic Multiparameter Multi Image Novel Apparatus), able to operate with sequences of RF frames in real time by means of its peculiar characteristic, which consists in the possibility to distribute any computational load between its hardware and software constituent parts. Images related to the structural organization of the carotid plaques were obtained by exploiting the capability of FEMMINA to extract a multitude of ultrasonic parameters and to present them simultaneously. The applicability of RULES in the clinical environment is assured by the FEMMINA real time data production rate.

2. INVESTIGATION METHOD

In echographic images, local organization of the microstructures, such as cells and micro vessels, give rise to a speckle effect. The speckle depends on the mechanical characteristics and spatial distribution of the microstructures. When an ultrasonic wave propagates through a soft tissue, an interaction between the mechanical energy of the wave and the local structure occurs, generating energy absorption, reflection and scattering. The energy propagated back toward the ultrasonic transducer constitutes the ultrasonic echo signal. The multitude of the echo wavelets, generated from the tissue structural elements, interferes in constructive and destructive manners on the receiving transducer surface, according to their propagation path differences. As a result, amplitude modulation occurs on the RF.

The amplitude of the echo signal is affected by the reflectivity of the local tissue microstructures, which is a function of their mechanical characteristics, i.e. density and elasticity. Therefore, spatial distribution and mechanical characteristics of tissue microstructure (i.e. the type and condition of the tissue under investigation) strongly affect the appearance of an ultrasonic signal.

The ultrasonic signal collected by the receiving transducer surface appears as a collection of summed wavelet echoes of different amplitude whose duration and frequency content are related to the bandwidth and center frequency of the incident ultrasonic pulse, respectively. Such a received signal is named radiofrequency (RF), or "raw" signal. Amplitude and phase information are contained in the RF echo signal.

It is hypothesized that the amplitude information is related to the distribution of mechanical impedance (density, elastic characteristics) of the backscattering medium and to the ratio of the sizes of the microstructure to the wavelength. Phase information, related to the interference, depends on mutual distances and geometrical organization of the tissue microstructure scatterers.

These interferences and reflectivity variations in the time domain are responsible for spectral amplitude modulation in the frequency domain. As a consequence, the preservation of the entire band of the RF signal is essential to **"**read" this amplitude spectrum shape, in order to gain further information for tissue characterization and differentiation purposes. Tissue imaging based on signal envelope extraction ignores part of the information contained in the RF echo signal and has limited use for tissue characterization.

In this scenario, we proposed the RULES method to provide a detailed description of the RF signal spectral content for gaining local microstructure information about tissue [8]-[11]. The aim of RULES is the real time differentiation of *in vivo* biological tissue type and its status for clinical diagnostics.

RULES produces a final image where the different structural organization of an investigated tissue is recognized and represented by different chromatic codes over a conventional B-Mode image. In particular, in this work RULES has been devoted to differentiation of the different plaque components.

The RF signal contains information about ultrasound–tissue interaction and it is necessary to employ a processing method capable of extracting the information in a meaningful way. Biological tissue can be seen as a distribution of non-regularly located scatterers which generate non-stationary signals. Consequently, the spectral content of the transmitted signal is locally modified and a Time-Frequency Representation (TFR) is required. After evaluating several kinds of TFRs, Wavelet Transform and in particular the Discrete Wavelet Packets Transform (DWPT), was chosen. The DWPT was realized by means of a parallel architecture of the Mallat algorithm and the third decomposition level without decimation was chosen as the best compromise between time and frequency resolution.

The first step of RULES method consists in the acquisition of the RF echographic frame. The RF signal of each track is decomposed into eight bands, derived from the third decomposition level, and eight sets of DWPT coefficients are produced. RULES allows local tissue characterization because it considers for each time instant (i.e. depth in the tissue) the eight DWPT coefficients whose behavior in the frequency domain was interpolated by means of a 4^{th} order polynomial. The polynomial coefficients a_0, a_1, a_2, a_3 and a_4 are used as Local EStimators (LES). The chosen order of the polynomial is a good compromise between the computational load and the fitting features. For each RF frame, five LES matrices are calculated.

An appropriate-sized rectangular window is translated step by step over all the LES matrices, in order to extract the histograms of the LES. For each step, the method extracts tissue information working on the window and a pixel of the final image is produced. The corresponding dimensions of such a pixel are defined by the values of the horizontal and vertical translations. Usually, the pixel dimensions are less large of those of the processing window.

The next processing step leads to individuate those portions of the RF frame that exhibit similar histogram characteristics for the same Local EStimator (LES). Each portion of the RF frame is characterized by peculiar histograms for each of the five LES. The shape of the obtained histograms is a signature of the structural organization of the tissue. As a consequence, tissue portion which produce histograms of similar shape can be considered acoustically homogeneous.

Similarity criteria are formulated for LES histograms by evaluating the following statistical parameters: maximum occurrence frequency, mean value, standard deviation and shape indices (kurtosis and skewness). For each of them a tolerance range must be considered. Different regions exhibiting statistical parameters with value belonging to the same range are considered homogeneous regions.

By defining "Configuration" as a particular set of value of these statistical parameters, several possible "Configurations" can be found to recognize different homogeneous regions, in the tissue under investigation.

Regions that are characterized by the same "Configuration" are considered homogeneous. The histological analysis of the investigated tissue allows a biological meaning to be given to the concept of "Configuration". In the RULES "learning phase", the "Configurations" are continuously improved till the matching with the histological analysis is reached.

The last step of RULES method concerns the visualization of the results. For each recognized "Configuration" a chromatic or grey code was superimposed on the conventional B-Mode image. The different chromatic codes correspond to homogeneous areas with different histological significance.

3. EXPERIMENTAL SET UP AND RULES LEARNING PHASE

The experimental work was carried out by using a FEMMINA [12] platform connected to a commercial echograph (Esaote S.P.A, AU3 equipment). A linear array probe (Esaote LA13 model) was employed. The frequency response of the probe presents a –6 dB bandwidth of 9 MHz and a 7.5 MHz center frequency.

The echographic RF frames were acquired through FEMMINA by using a sampling frequency of 40 MHz. Each RF signal of the acquired frames was processed by the RULES algorithm distributed between the hardware and software components of FEMMINA, in order to maintain a data production rate of 43 frames per second imposed by the echograph.

The RULES learning phase was performed by selecting 22 patients affected by different degrees of carotid stenosis requiring surgery. For each patient, two experimental phases were performed. The first consisted in *in vivo* acquisition before the surgical operation and the second, which constituted the learning phase, was performed *in vitro* on the removed carotid specimens. The experimental protocol scheduled the postoperative histopathological examination of the removed specimen to evaluate the relative content of calcification, fibrous tissue, necrosis and fiber cap in order to produce histological maps.

The specimen**,** without being fixed in formalin**,** was placed in a tank filled with normal saline and fixed by threads to a metallic frame in a particular position in order to preserve the local spatial reference of the slices employed for histological mapping. The specimen was ultrasonically scanned in parallel planes moving the array probe with a mechanical system.

A total number of 440 *in vivo* and 187 *in vitro* RF frames were produced.

The learning phase for carotid characterization was performed on 187 *in vitro* frames by comparing the results with the related 187 histological maps. The "Configurations" were created by using these data sets. Different kinds of structural organizations were recognized and mapped with their own chromatic code over the B-Mode image.

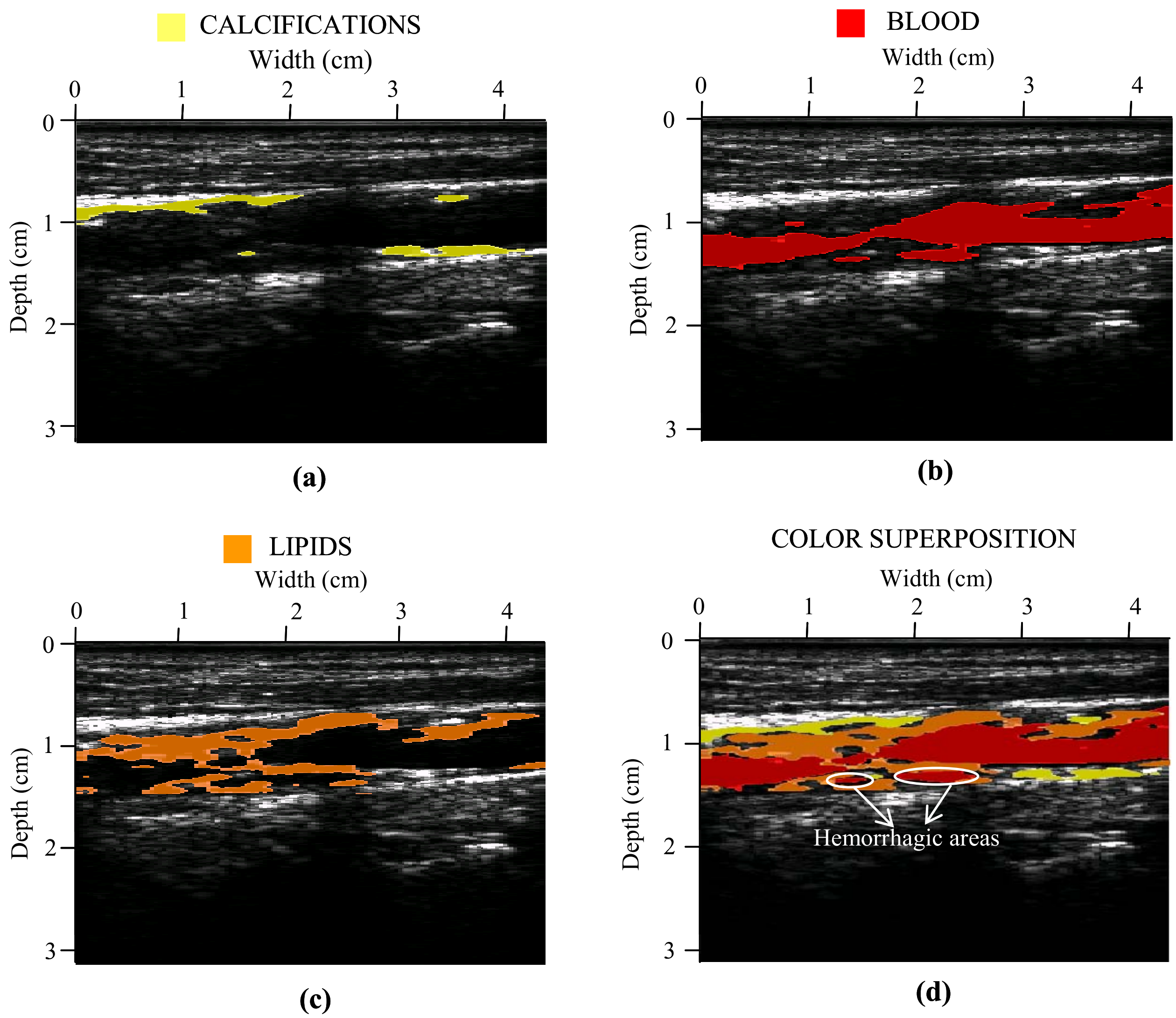

Fig. 1. Final RULES images of an *in vivo* carotid longitudinal section: a) calcific detection by using CONFIGURATION C; b) blood presence detection by using CONFIGURATION B; c) lipids and necrosis detection by using CONFIGURATION L; d) composite image of the three detected structures represented simultaneously with circled hemorrhagic areas.

Then, by comparing the homogeneous image portions thus produced with the corresponding zone of the histological maps, it was possible to drive the search for the most appropriate set of RULES parameters for plaque structure differentiation and blood detection. The learning phase was closed when a perfect matching was found between RULES and histology results.

In particular, two typical sets of parameters defined as "Configuration L" and "Configuration C" were produced for lipids and calcification detection, respectively.

In a previous work, the authors detected blood presence by analyzing the signal spectrum backpropagated from the blood constituent particles, without using the Doppler effect [8].

A specific RULES "Configuration", labeled "Configuration B", for blood presence detection was identified. The conjunction of the three "Configurations" can furnish a complete carotid vessel characterization, where the information on plaque structure is enriched with indications regarding the residual lumen dimension. Moreover, the identification of hemorrhagic zones inside the plaques, where no blood motion exists, becomes a possible feature.

These three "Configurations" were then tested on the *in vivo* acquisitions previously collected. An example is shown in Fig. 1 where a clinical relevant case is considered. In fact, blood presence inside the lipid plaque can be observed by analyzing the blood (Fig. 1b) and lipids (Fig 1c) detection images. The histological examination confirmed the breaking of the lipid plaque fiber cap in two different zones. The two hemorrhagic areas are highlighted with circles in the composite image (Fig. 1d).

4. CONCLUSION

A novel investigation method for tissue differentiation, RULES, has been developed. The promising results for tissue differentiation and pathology detection in carotid plaques in humans produced by the method learning phase were presented.

The learning phase of RULES on *in vitro* specimens supported by histological analysis led to the definition of two prominent "Configurations", able to distinguish lipid and necrotic structures from calcifications. Starting from previous experimental work, a further "Configuration" for blood presence detection has been defined. It has been demonstrated that the three defined "Configurations" well classify all the 22 patients involved in the learning phase. Considering these results, the three RULES "Configurations" are currently employed in clinical diagnosis in the Department of Vascular Surgery of the University of Florence, in order to check for specificity and sensitivity. Currently RULES follows a clinical validation protocol in the Department of Vascular Surgery of the University of Florence in order to check its specificity and sensitivity. The protocol recruits 100 patients with severe atherosclerosis which must be subjected to endartectomy and 50 healty patients.

In the initial phase of the protocol, 20 healthy patients were investigated and a specificity of 95% has been observed.

5. ACKNOLEDGMENT

The authors have performed the clinical test of RULES with Prof. C. Pratesi's group at the Department of Vascular Surgery of the University of Florence. The histological analyses were furnished by the Department of Human Pathology and Oncology (Prof. F. Gori) of the University of Florence.

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